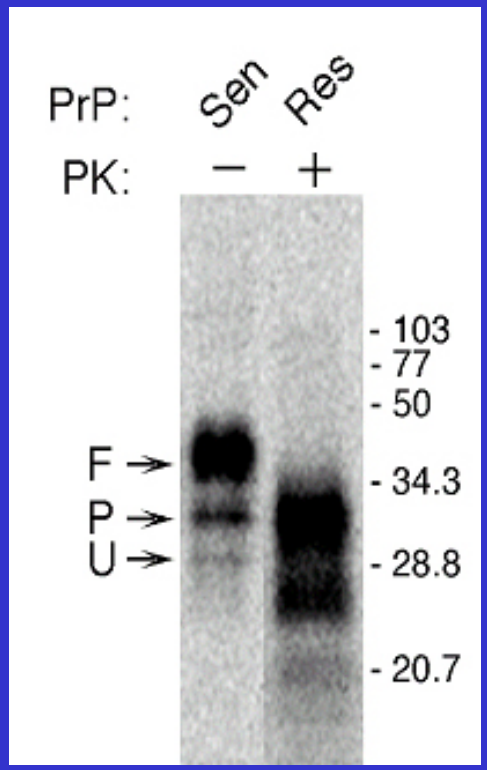
Prion Protein (PrP) and TSE Disease

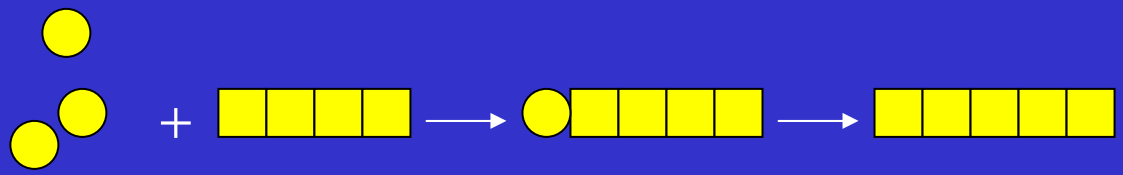


Normal PrP (PrP-sen)

- Proteinase K sensitive
- Detergent soluble
- Expressed in many different tissues
- Primarily alpha-helix/ loop structure

Abnormal PrP (PrP-res)

- Proteinase K resistant
- Detergent insoluble, aggregated
- TSE specific (CNS, LRS)
- Mostly beta-sheet structure



PrP-sen + PrP-res → Accumulation and disease

Susceptibility of different cell types to TSE infection

Susceptible Cell Lines

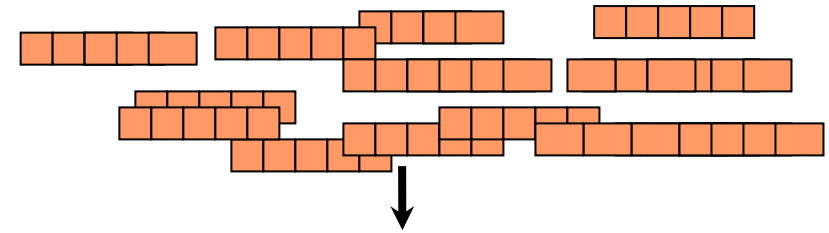
- Neuronal Cells
  - –Mouse neuroblastoma (N2A, MNB)
  - –Mouse hypothalamic (GT-1)
  - –Rat pheochromocytoma (PC-12)
  - –Hamster or mouse brain cells
- Non-Neuronal Cells
  - –Mouse L-fibroblasts
  - –Rabbit kidney epithelial (modified)
  - –Mouse Schwann (MSC-80)

Non-Susceptible Cell Lines

- Neuronal Cells
  - •Human neuroblastoma (p12-3)
  - •Human glioma (HJC-15)
- Non-Neuronal Cells
  - •Human embryo lung fibroblasts (WI-38, MDC-5)
  - •Human embryo brain fibroblasts
  - •Human embryo kidney
  - •Chinese Hamster Ovary
  - •AGMK (BSC-1)

⟶ So far no human or bovine cell lines

TSE infection of tissue culture cells is often inefficient



High Multiplicity of Infection

Cell type

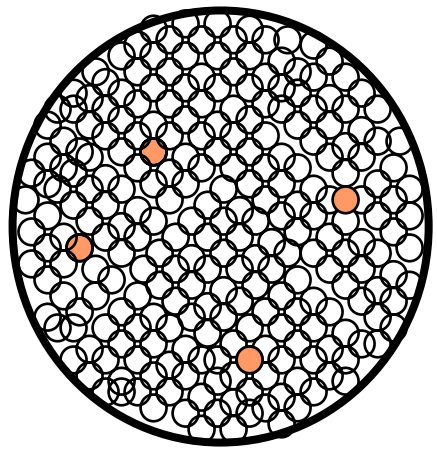
PrP sequence, amount, conformation

Single Cell

Passage

Dilution

Persistence



<1%-60% PrP-res positive cells

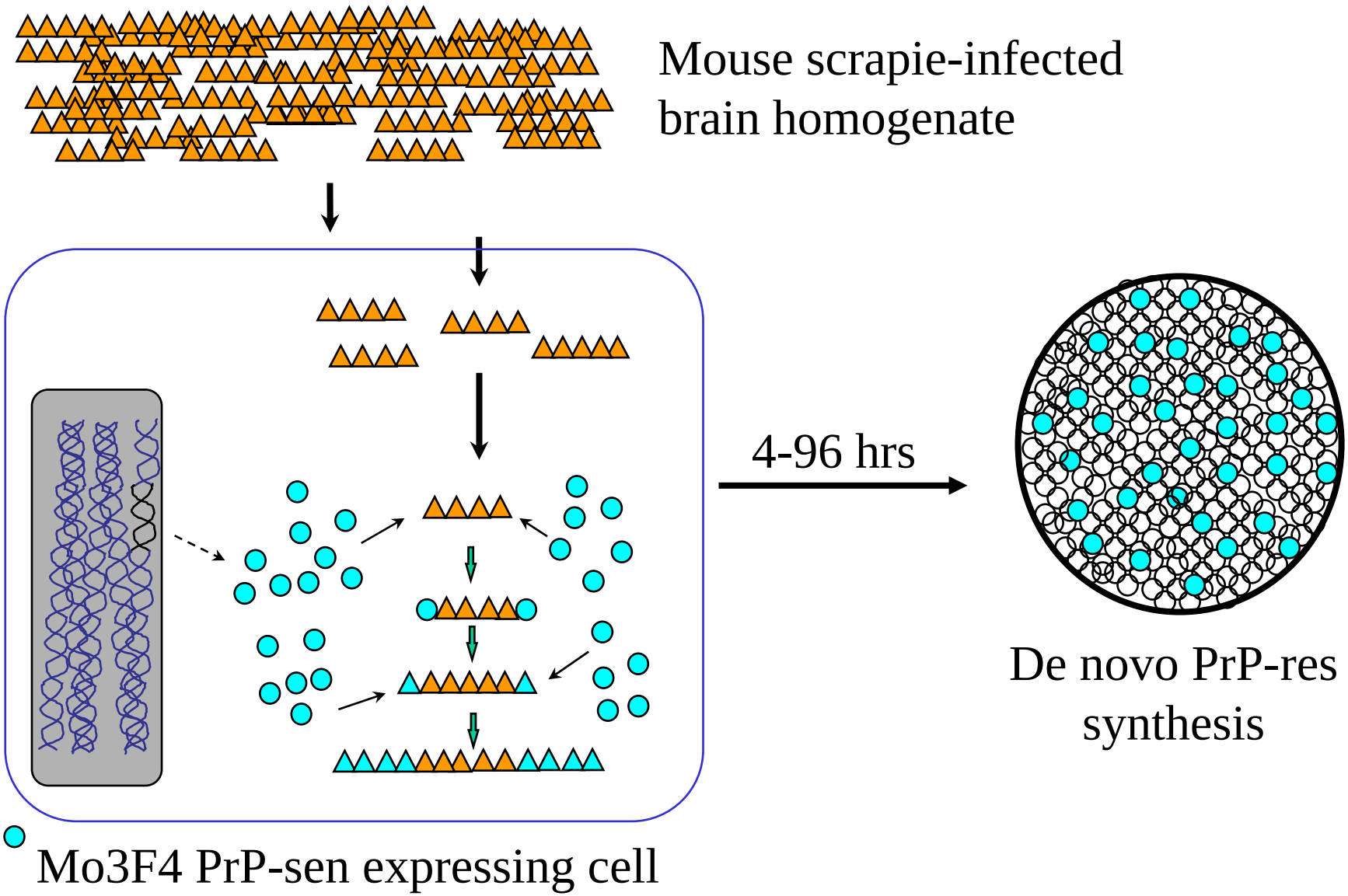
Possible factors influencing susceptibility of tissue culture cells to TSE infection

- Cell type (neuronal vs non-neuronal)
  - Ability of cell to support PrP-res formation
- Strain and species of TSE infectivity
- PrP-sen expression

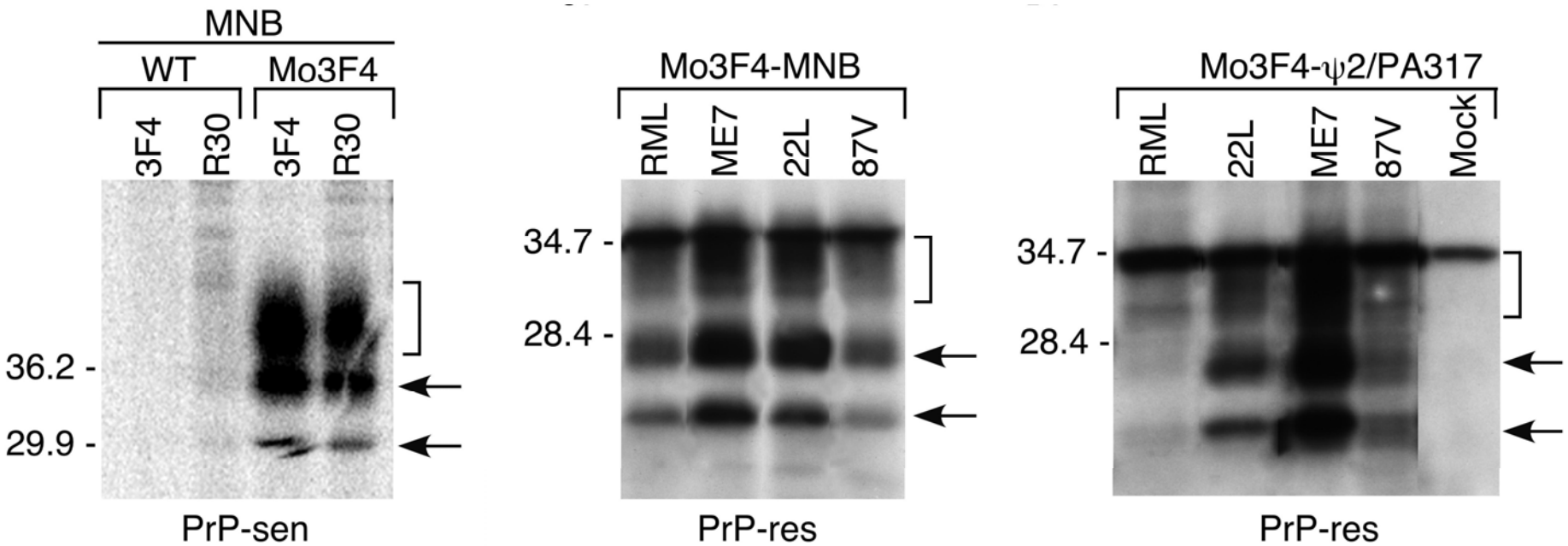
a) Expression level

b) Amino acid sequence (species specificity)

Overlay assay for de novo PrP-res formation

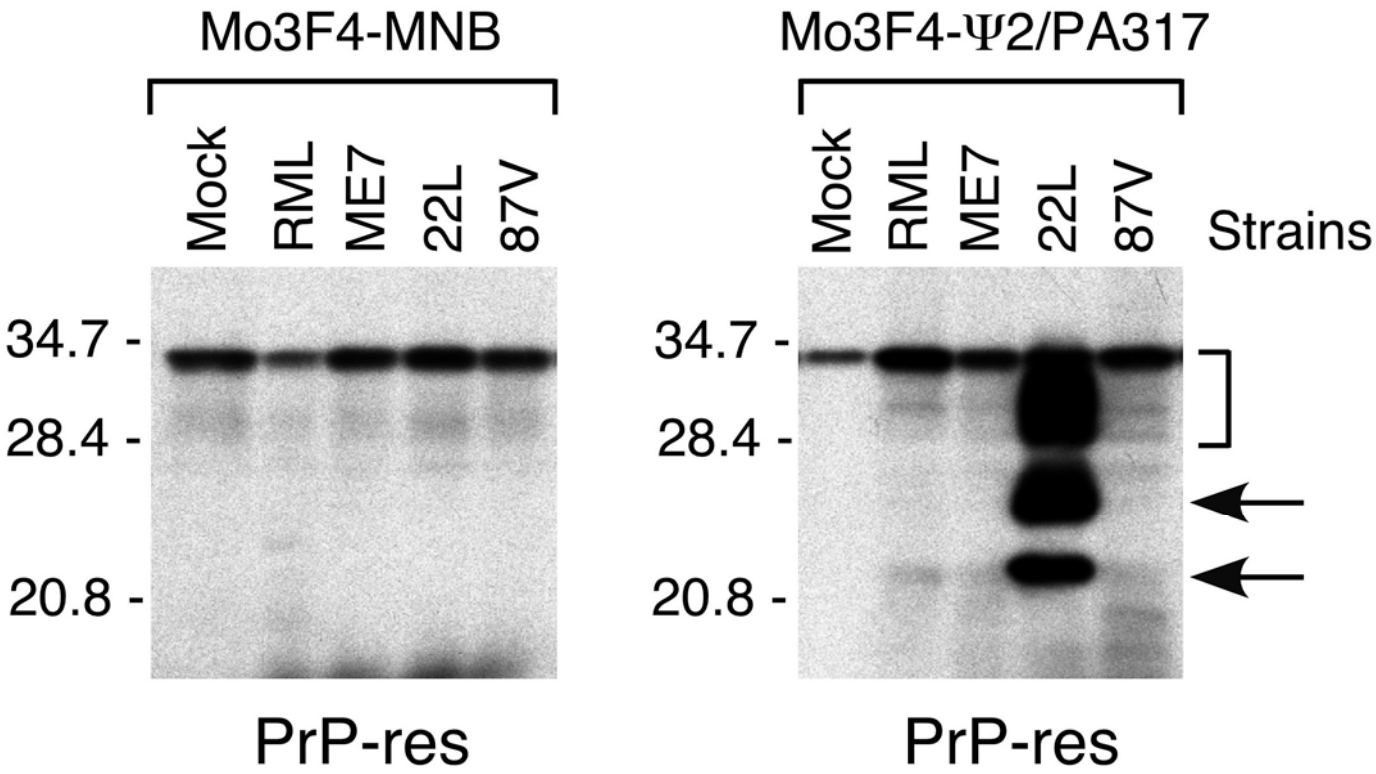


Both neuronal and non-neuronal cells can support PrP-res formation



J. Biol. Chem. 279:29218 (2004)

Acute PrP-res formation does not necessarily lead to persistent PrP-res formation



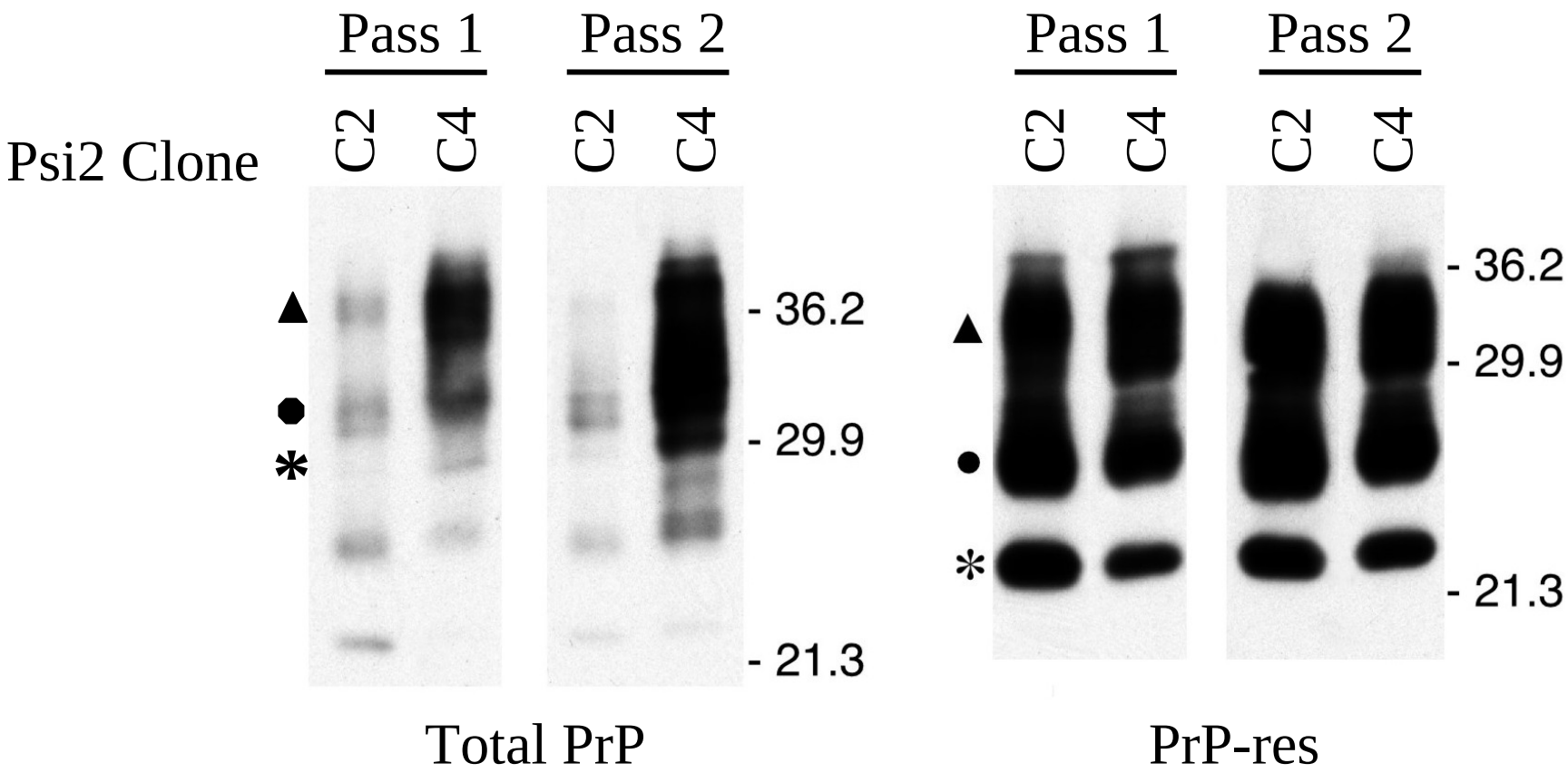
→ Fibroblast cells are susceptible to TSE infection

Mouse bioassay of cells exposed to TSE infectivity

	Mouse Cell Type	
Strain	Mo3F4-MNB	Mo3F4-Psi2/PA317
22L	0/6 (>755dpi)	8/8 (285±40dpi)
RML	0/4 (>755dpi)	1/7 (624, >753dpi)
ME7	0/7 (>755dpi)	0/5 (>753)
87V	0/4 (>755)	0/6 (>755)

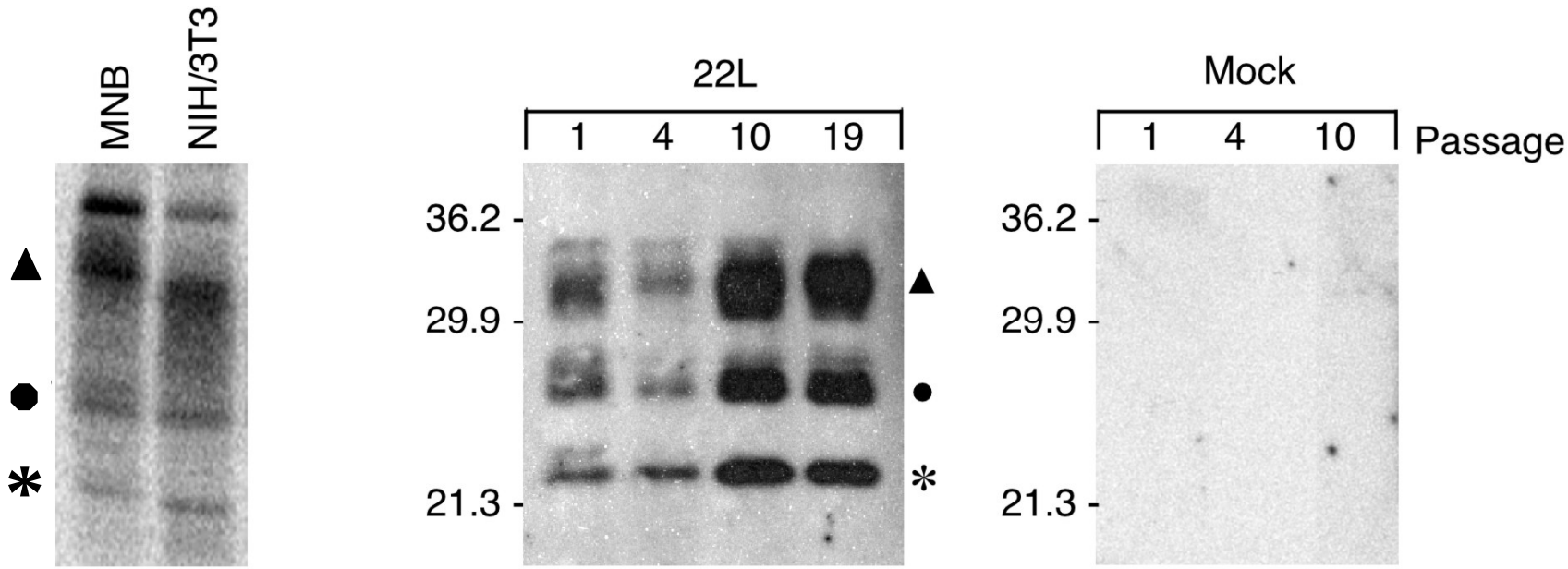
J. Biol. Chem. 279:29218 (2004)

PrP-sen expression level does not predict the susceptibility of a cell to TSE infection



J. Infect Dis. 189:431 (2004)

NIH 3T3 mouse fibroblast cells can be persistently infected with mouse scrapie

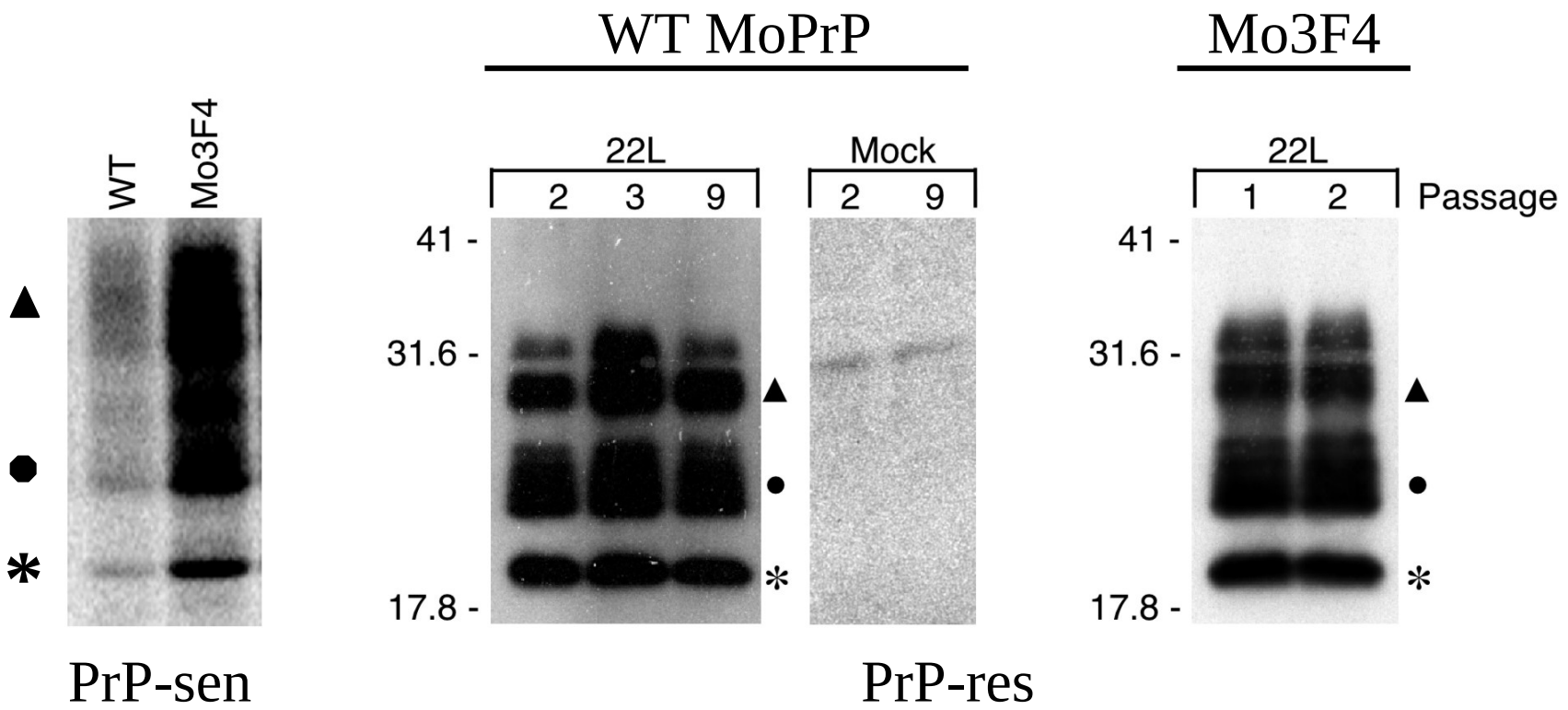


PrP-sen

PrP-res

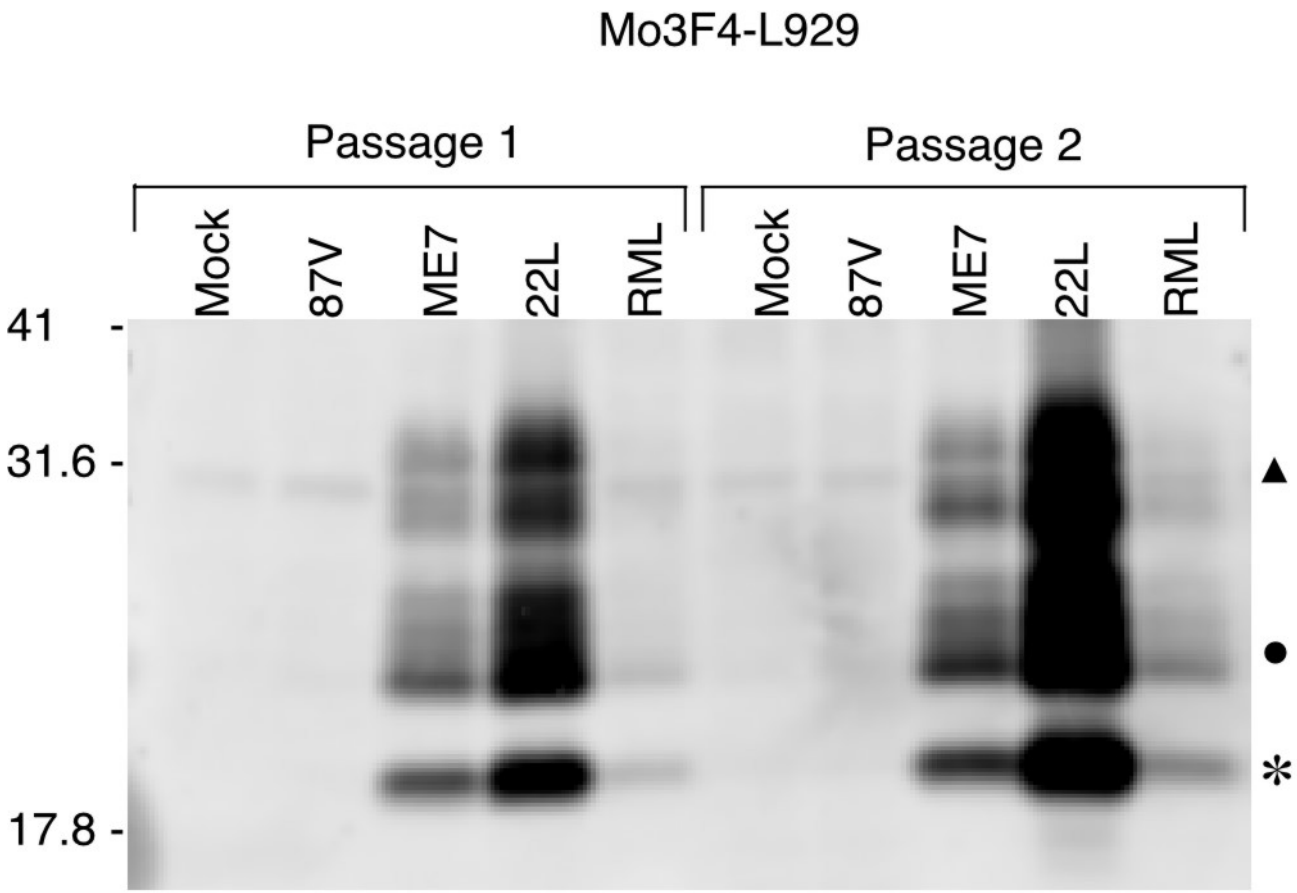
J. Infect Dis. 189:431 (2004)

Mouse L929 fibroblast cells can be persistently infected with mouse scrapie



J. Infect Dis. 189:431 (2004)

Multiple mouse scrapie strains can persistently infect mouse L929 fibroblasts



PrP-res

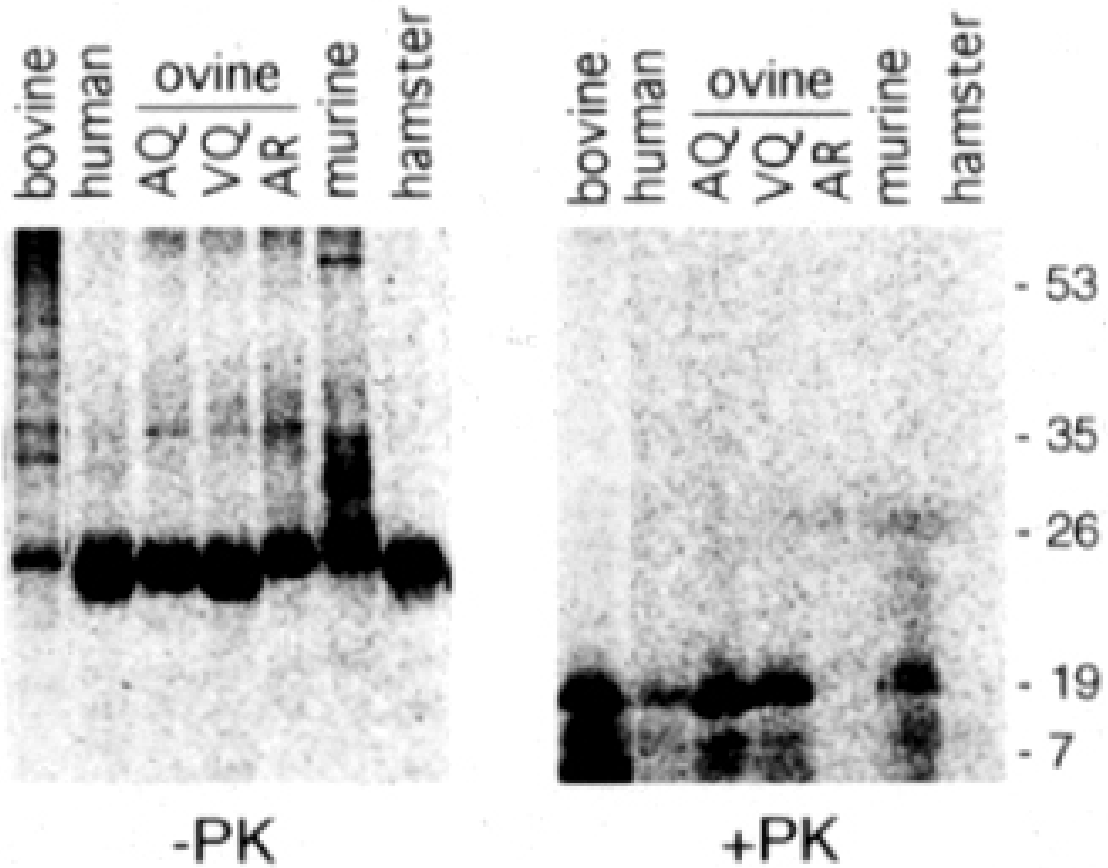
J. Infect Dis. 189:431 (2004)

Conclusions

- Cannot predict susceptibility to TSE infection based upon:
  - Acute PrP-res formation
  - Expression level of PrP-sen
  - Cell type
    - Neuronal vs non-neuronal
    - Cell passage history
  - Strain of TSE agent

- Factors that likely influence susceptibility to TSE infection:
  - PrP-sen must be expressed
  - PrP-sen amino acid sequence homology with PrP-res [Villette et al. PNAS 98:4055 (2001)]

PrP^{BSE}-induced conversions of various species' [^{35}S] PrP-sen molecules



Nature 388:285 (1997)

Implications

- Without direct assessment of susceptibility to TSE infection, it is largely invalid to assume that a particular cell type will be resistant to a TSE agent

- To assay the susceptibility of a given cell type to TSE infection:
  - Expose cells to TSE agent of interest at both high and low multiplicities of infection
  - Assay cells for PrP-res formation over multiple passages
  - If PrP-res is not detected:
    - Assay cells for infectivity using the appropriate in vivo bioassay

Acknowledgements

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